REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance.

Status of the Claims and Formal Matters

Claims 1-3, 6-29, and 32-44 are currently pending in this application. Claims 16-21, 23, and 35-44 were previously withdrawn as allegedly being drawn to a non-elected invention. Applicants assert the right to reclaim withdrawn or cancelled subject matter in co-pending applications. By this paper, Claims 1, 14, 22, and 34 have been amended, without prejudice, and solely to expedite prosecution pursuant to the U.S. Patent and Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). No new matter has been introduced by these amendments. Support for the amendments to the claims can be found throughout the specification as originally filed, particularly at page 12, lines 1-17; Table 3 at page 25, lines 1-2; page 21, lines 1-4; and page 8, lines 20-31.

Rejections under 35 U.S.C. §103(a)

Claims 1-3, 6-14, 22, 24, 25, 32, and 33 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths (U.S. Patent Application No. 20020119459; hereinafter "Griffiths") in view of Wangh et al. (U.S. Patent Application No. 20040053254; "Wangh") and further in view of Strizhkov et al. (Biotechniques (2000) 29: 844-857; "Strizhkov"). According to the Office Action, it allegedly would have been *prima facie* obvious to those skilled in the art to combine the method of Griffiths for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion on the surface of a bead with the method of Wangh for non-symmetric PCR using primers at unequal concentrations, since the Wangh method is allegedly suited for amplifications that utilize small reaction volumes and very low copy numbers of target sequences. The Office Action further contends that it would allegedly have been obvious to utilize two populations of a first primer -- one attached to a solid surface and one in solution, since the primer in solution can perform the initial rounds of amplification on the target nucleic acid, while subsequent rounds of amplification of the resulting extension products can be further amplified in the solid phase for detection using the immobilized forward primer and the

labeled reverse primer present in solution according to Strizhkov. Applicants respectfully traverse this rejection.

Applicants previously argued in the response filed April 1, 2008 that the combination of Griffiths, Wangh, and Yu failed to describe the claimed invention, and on page 19 first paragraph the office action states "The Examiner agrees that neither Yu nor Wangh teach two distinct populations of a first primer, one in solution and one immobilized on a solid support".

The present Office Action now alleges that the replacement of Yu with Strizhkov cures the deficiencies when used in combination with Wangh and Griffiths. In particular, the Office action states at page 19 that, "[i]t is obvious that the methods of Wangh using unequal concentrations of solution-based primers can be combined with the solid phase methods of Strizhkov using a forward primer present in both the solid and solution phases, with the second primer in solution, which is labeled in the methods of Strizhkov, chosen as the more abundant primer species in solution."

However, Applicants respectfully assert that the presently claimed invention is distinguishable from the combined teachings of Wangh, Griffiths, and Strizhkov and have amended claims 1, 22, and 34 to further clarify this point. In particular, amended claims 1, 22, 34, and claims depending therefrom now recite that the number the molecules of the first population of the first primer species immobilized on the bead are each present in greater numbers within the aqueous microreactors than the number of molecules of the of the second population of the first primer species in the reaction solution. Strizhkov describes the exact opposite situation, where the population of primer species in solution is at least equal to or greater than the population of the same primer species immobilized in the gel. Thus, Strizhkov would be considered by the skilled artisan as teaching away from the presently claimed invention.

Support for the amendments are specifically recited in the specification, but requires the conversion of some of the units of measurement to gain the full appreciation of their meaning. Applicants assert that such unit conversion is well within the knowledge and capabilities of those ordinarily skilled in the art. For example, page 12, lines 1-17 of the instant specification describe that the beads have between 10 and 30 million primers immobilized thereon (first population of the first primer species), and page 25, lines 1-2 (specifically table 3) describes the volumes and concentrations of the first and second primer species added to the reaction mixture. In the

present example, the claimed first primer species present in the reaction solution (i.e. the second population) is represented as "Primer MMP1b" where the final primer concentration in the reaction solution is 0.078 μM.

One of ordinary skill in the art would understand how to use these numbers to calculate the number of primer molecules present in a given volume of solution in the microreactors. For example, a given aqueous microreactor will contain a volume of reaction solution that is equal to $4/3(\pi R^3)$, where R is the radius of the microreactor. Therefore, if a given microreactor is 50 μm in diameter (R = 25 μm) the volume of the reaction solution in the droplet is 6.5×10^4 μm^3, which is equivalent to 6.5×10^{-5} μL (given that 1 μm^3 = 1 femtoliter, and 1×10^9 femtoliters = 1 μL). One of ordinary skill would further understand that a 0.078 μM concentration is equivalent to 0.078 pmole/μL, and thus the 6.5×10^{-5}μL volume within the microreactor will contain 5.1×10^{-5} pmoles, which given that there is 6.02×10^{11} molecules/pmole, equates to 3.06×10^6 primer molecules in a 50 μm droplet. In other words, the specification describes and clearly supports that a 50 μm microreactor contains about 3,060,000 molecules of the second population of first primer species in the reaction solution which is substantially less than the described 10 – 30 million molecules of the first population of the first primer species immobilized on the bead.

Those skilled in the art will further appreciate that the claimed beads in the microreactors occupy a volume of space in the droplet which would otherwise be occupied by the reaction solution. It is understood that some types of beads that could be employed in the presently claimed invention have a measure of porosity, and could absorb some volume of solution into the bead. Thus, subtracting the absolute numerical volume occupied by the bead from the internal volume of the droplet for the calculation of primer numbers would not be entirely accurate. However, regardless of the degree of porosity, a bead still occupies a measure of volume within the droplet, and therefore the actual number of the first population of the first primer species would be less than the 3 million molecule number recited above. This further emphasizes the difference between the primer numbers of the two populations.

Applicants respectfully assert that the combination of Wangh, Griffiths, and Strizhkov do not describe that the number of molecules of a first immobilized population of a primer species is greater than the number of molecules of a second population of the same primer species in solution. In fact, Strizhkov specifically teaches that the number of molecules of a population of the primer species in the reaction solution is at least equal to, or greater then the number of

molecules of a immobilized population of the same primer species. For example, Strizhkov clearly describes that there is about 1 pmole of primers immobilized in the gel substrate (see page 845, middle col 3rd paragraph) and also describes that there is 1-10 pmoles of the same primer added to the reaction solution (see page 845, right col 3rd paragraph near bottom). Therefore, in view of the foregoing arguments, Applicants respectfully assert that the combination of Griffiths, Wangh, and Strizhkov fail to teach or disclose all of the instant claim limitations with a reasonable expectation of success. The rejection should be withdrawn.

Claims 15 and 26-29 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Wangh and Strizhkov and further in view of Jurinke et al. (U.S. Patent No. 6,303,309; "Jurinke"). The Office Action concedes that none of Griffiths, Wangh, or Strizhkov teach a method for amplifying one or more nucleic acids wherein more than 10,000 or at least 1,000,000 amplification copies of each target nucleic acid molecule are bound to each bead. None of Griffiths, Wangh, or Strizhkov teach a method for amplifying one or more nucleic acids wherein the beads are Sepharose beads. Jurinke allegedly teaches a method of purification of biotin-labeled PCR products by complexing the products to a solid support containing a biotin-binding compound such as streptavidin immobilized on the surface, including agarose, Sepharose, or magnetic beads. Jurinke allegedly teaches immobilization of 100 pmol of biotinylated oligonucleotides to 50 µl of streptavidin-coated magnetic beads, which the Office Action states as representing about 1 million molecules bound per bead. The Office Action contends that it would allegedly have been obvious to the skilled artisan to combine the methods of Griffiths, Wangh, and Strizhkov for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using non-symmetric PCR with that of Jurinke for purification of PCR products using solid supports such as magnetic or Sepharose beads, since the use of such beads allows further purification and extensive washing to remove all excess reaction components prior to final recovery of the PCR product. Applicants respectfully traverse.

As discussed elsewhere in this Response, the combination of Griffiths, Wangh, and Strizhkov do not render the instant claims unpatentable, because they fail to teach or disclose all of the instant claim limitations with a reasonable expectation of success, particularly a first immobilized population of a primer species that is greater than the second population of the same primer species in solution. Jurinke does not cure these deficiencies. Jurinke relates to a method of dissociating complexes of biotin and biotin-binding compounds like streptavidin, but does not

disclose methods of amplifying nucleic acids on a solid phase support such as a bead, using a number of molecules of a first immobilized population of a primer species that is greater than the number of molecules of a second population of the same primer species in solution as claimed. Because none of Griffiths, Wangh, Strizhkov or Jurinke teach or disclose all of the instant claim limitations, Applicants respectfully contend that *prima facie* obviousness under §103(a) has not been met. This rejection should be withdrawn.

Claim 34 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths, Wangh, and Strizhkov and further in view of Nakano et al. (J. Biotechnol. (2003) 102: 117-124; "Nakano"). The Office Action argues that it would allegedly have been *prima facie* obvious to those skilled in the art to combine the methods of Griffiths, Wangh, and Strizhkov for amplifying nucleic acids on a bead within a microcapsule, such as a water-in-oil emulsion, using non-symmetric PCR with that of Nakano, since Nakano allegedly teaches a method for amplifying multiple nucleic acids in water-in-oil emulsions that is adaptable to the methods of Griffiths, Wangh, and Strizhkov for amplifying multiple nucleic acid targets of different sizes in an emulsion containing a bead using a non-symmetric PCR process, since the products can allegedly be purified simultaneously on the same bead and later separated by sizing methods or simply analyzed by gel electrophoresis. Applicants respectfully traverse.

For reasons discussed herein, Griffiths, Wangh, and Strizhkov, whether considered individually or in combination, are deficient under §103(a) for failing to teach or disclose all of the instant claim limitations. Nakano relates to single-molecule PCR using a water-in-oil emulsion, wherein limiting dilutions of the DNA template were used to create conditions where one molecule of template was amplified inside a droplet comprised of silicone oil, Triton X-100, and PCR buffer. Nakano does not remedy the defects of Griffiths, Wangh, and Strizhkov, because Nakano does not teach or disclose methods of amplifying nucleic acids on a solid phase support such as a bead, using a first immobilized population of a primer species is greater than the second population of the same primer species in solution as claimed. Because none of Griffiths, Wangh, Strizhkov or Nakano teach or disclose all of the instant claim limitations, Applicants respectfully request that the §103(a) rejection over this combination of references should be withdrawn.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Applicants believe no additional fees are due with the filing of this Response. However, if any additional fees are required or if any funds are due, the USPTO is authorized to charge or credit Deposit Account Number: **50-0311**, Customer Number: **35437**, Reference Number: **21465-508 UTIL**.

Respectfully submitted,

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